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Aging-induced changes in microstructure and water distribution in fresh and cooked pork in relation to water-holding capacity and cooking loss – A combined confocal laser scanning microscopy (CLSM) and low-field nuclear magnetic resonance relaxation study

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Abstract

Confocal laser scanning microscopy (CLSM) and low-field nuclear magnetic resonance (LF-NMR) relaxometry were combined to characterize microstructural changes and water distribution in fresh and cooked pork during an aging period of 14 days. At day 1 (24 h *postmortem*) a few muscle fibres, which appear swollen, were observed in both fresh and cooked meat. An identical microstructure was still apparent after 14 days, however, the number of muscle fibres showing distinguished characteristics was found to increase throughout the aging period. Hence, it was apparent that during aging the individual fibres swell and disintegrate at different rates. Development in water-holding capacity (WHC) was followed during the aging period using gravimetric methods, and an increase in the WHC in the fresh meat was observed, which resembled the amount of extramyofibrillar water measured by NMR relaxometry (T_{22} population). This was consistent with the CLSM images, as a substantial increase in the number of myofibrils that appeared swollen, capable of holding more water, was observed during aging. In the cooked meat the width of the T_{21c} population, reflecting the myofibrillar water in the cooked meat, was seen to decrease during the entire storage period, which corresponds to the development of a more homogeneous structure. In the CLSM data a continuous degradation during the storage period was observed, which could resemble a shift to a more homogeneous structure. Comparison of CLSM of transverse sections of fresh and cooked pork revealed a pronounced shrinkage of muscle fibres upon cooking. This resulted in large gaps between the cooked muscle fibres, which also was visible as shrinkage at the level of the individual myofibrils. This pattern was also reflected in the NMR relaxation data. The cooking-induced shrinkage of the myofibrils occurred concomitantly with a decrease in the amount of intermyofibrillar water within the individual fibre and an increase in the larger extramyofibrillar spaces between fibres, i.e. water is expelled from the myofibrillar matrix upon cooking. Accordingly, the present study demonstrated that the use of CLSM together with NMR relaxometry can provide further information on the relationship between structural characteristics of meat and resultant water distribution.

Keywords: NMR T_2 relaxation; Muscle fibres; Myofibrils; Meat structure; Aging; Proteolysis; WHC; Drip loss; Cooking

1. Introduction

Water-holding capacity (WHC) of meat is of great importance in the meat industry, as it affects both economic and sensory attributes of the meat (Oeckel, Warnants, & Boucqué, 1999). The structural organization of the muscle proteins is decisive for the distribution of the water within the meat, and thereby directly affects the WHC characteristics of meat (Huff-Lonergan & Lonergan, 2005; Kristensen & Purslow, 2001; Melody et al., 2004; Morrison, Mielche, & Purslow, 1998). Consequently, a

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thorough understanding of the structural features and simultaneous water distribution during production and processing of meat is important to understand the characteristics determining WHC.

The microstructure of meat has in the past been studied in detail using several traditional microscopic methods, e.g. light, electron and fluorescence microscopy (Borchert, Greaser, Bard, Cassens, & Briskey, 1967; Guignot, Vignon, & Monin, 1993; Maher et al., 1998; Morrison et al., 1998; Offer & Cousins, 1992; Offer & Knight, 1988; Schäfer, Rosenvold, Purslow, Andersen, & Henckel, 2002; Swatland & Belfry, 1985; Voyle, 1981). However, in all these microscopic approaches the specimens for analysis were fixed or manipulated in other ways. This always raises questions regarding formation of artefacts as a consequence of such fixation procedures.

Confocal laser scanning microscopy (CLSM) is an alternative technique for studying structures, which evades many of the above mentioned problems as it does not require any rough fixation procedures, and simultaneously it allows improved imaging possibilities for thicker specimens, which is of importance in e.g. the study of meat structure.

CLSM has been successfully applied to the study of localization of spoilage bacteria within the protein environment of fresh meat (Delaquis, Gariépy, & Montpetit, 1992; Prachaiyo & McLandsborough, 2000) and structural features of meat gels, heterogeneous emulsions, vegetables, bread and dairy products (Auty, Twomey, Guinee, & Mulvihill, 2001; Dürrenberger, Handschin, Conde-Petit, & Escher, 2001; Lorén, Hagslätt, Nydén, & Hermansson, 2005; Verbeken, Neirinck, Van Der Meeren, & Dewettinck, 2005). However, to the authors' knowledge CLSM has not yet been used to study structural features of transverse sections of fresh meat.

During recent years an extensive effort to obtain basic information about water mobility and distribution in meats has been carried out using low field nuclear magnetic resonance (LF-NMR) relaxometry (for an extensive review, see Bertram & Andersen, 2004). This has contributed to a better understanding of several factors of importance for the WHC of meat, e.g. influence of the rate and extent of *postmortem* processes, cooling and heating.

Several studies have reported an improved WHC of meat as a function of aging (Boakye & Mittal, 1993; Joo, Kauffman, van Laack, Lee, & Kim, 1999; Kristensen & Purslow, 2001; Oreshkin, Borissowa, Permjakow, & Burstein, 1989). This improvement in WHC has been proposed to be due to proteolytic degradation of cytoskeletal proteins, which subsequently enables swelling of the myofibrils and is expected to allow the meat structure to retain myowater (Huff-Lonergan & Lonergan, 2005; Kristensen & Purslow, 2001; Melody et al., 2004; Morrison et al., 1998). However, the exact changes in the structure during aging and how these influence the distribution of the water within the meat are far from understood in relation to the WHC and subsequent cooking loss.

The aim of the present study was to use CLSM for the first time to characterize microstructural changes in transverse sections of fresh and cooked pork during aging and to elucidate the effect of these microstructural changes in relation to water distribution in the meat using LF-NMR relaxometry.

2. Materials and methods

2.1. Meat sampling

Six DLY cross bred pigs (Duroc boar crossed with Danish Yorkshire/Danish Landrace sow) with a live weight of approximately 80–100 kg at the time of slaughter were included in the study. The pigs were slaughtered at the experimental abattoir at Research Center Foulum. The pigs were stunned by 80% CO_2 for 3 min, exsanguinated and scalded at 62 °C for 3 min. Cleaning and evisceration of the carcasses were completed within 30 min *postmortem*. At 45 min *postmortem*, the carcasses were split and transferred to a room with a temperature of 12 °C, and after 60 min they were stored in a cooling room at 4 °C.

From the third rib curvature of the right *m. longissimus dorsi* (LD), approximately 24 cm was cut from two pigs and approximately 40 cm from four pigs 24 h *postmortem* (day 1).

From each of the two 24 cm LD cuts, 15 samples of approximately 1 × 1 cm in cross-sectional area and approximately 5 cm long (~5 g) were cut along the fibre direction on day 1. The samples were placed in cylindrical glass tubes and stored at 4 °C for up to 14 days *postmortem*. During the aging period confocal microscopic examination of the meat was carried out on day 1, 2, 4, 7 and 14. Three meat samples were examined on each of the 5 days.

From each of the four 40 cm LD cuts, five samples approximately 8 cm long were cut, and the samples were stored at 4 °C for up to 14 days *postmortem*. NMR measurements and determination of WHC were carried out on day 1, 2, 4, 7 and 14. The order of sampling was from the third rib curvature towards the head for two pigs, and the sampling was in the opposite direction for the remaining two pigs.

2.2. pH measurements

pH was measured at the third rib curvature of LD 24 h *postmortem* with an insertion electrode (Radiometer, Copenhagen, MeterLab, PHM201). The temperature used to calibrate the electrode was 4 °C.

2.3. Determination of water-holding capacity (WHC)

Measurement of WHC was performed using two complementary methods: the Honikel bag method (Honikel, 1998) and a centrifugation method described by Kristensen and Purslow (2001).